DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
§ 72-4(c)

1. **CHEMICAL:** Novaluron PC Code No.: 124002

2. **TEST MATERIAL:** Rimon Technical Purity: 99.9%
[Difluorophenyl-^{14}C(U)]Rimon >97%

3. **CITATION:**

Authors: Lima, W.

Title: Novaluron - Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*)

Study Completion Date: February 14, 2002

Laboratory: Springborn Laboratories, Inc.
790 Main Street
Wareham, MA 02571-1075

Sponsor: Markhteshim Agan of North America, Inc.
551 Fifth Avenue, Suite 1100
New York, NY 10176

Laboratory Report ID: 11742.6143

MRID No.: 45638212

DP Barcode: D285479

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan **Date:** 4/1/03

APPROVED BY: Christie E. Padova, B.S., Staff Scientist, Dynamac Corporation

Signature: Christie E. Padova **Date:** 4/1/03

5. **APPROVED BY:** Bill Evans, Biologist, OPP/EFED/ERB - I

Signature: William Evans **Date:** 4/20/03

6. STUDY PARAMETERS:

Scientific Name of Test Organisms: *Americamysis bahia*

Age of Test Organism: Neonates (≤ 24 hours old)

Definitive Test Duration 28 days

Study Method: Flow-through

Type of Concentrations: Mean-measured

7. CONCLUSIONS:

In a 28-day life cycle test, *Americamysis bahia* neonates were exposed under flow-through conditions to mixture of [difluorophenyl-^{14}C(U)]Rimon plus unlabeled Rimon Technical at mean-measured concentrations of <0.39 (LOQ; negative and solvent controls), 7.4, 14, 26, 60, and 120 ng/L. Nominal test concentrations were 0 (controls), 8.2, 16, 33, 65, and 130 ng/L. Prior to sexual maturity and pairing, there were 60 mysids/level. At Day 14, 20 pair/level were isolated for individual matings; the remainder of first-generation mysids were group retained. First-generation mysids were observed for mortality and signs of abnormal behavior once daily throughout the study. Once daily during the reproduction period, second-generation mysids were counted and discarded. Data endpoints included terminal percent survival of first-generation mysids (Day 28; combined sexes), number of young produced per female per reproductive day, and dry weight and length of surviving first-generation mysids (Day 28; gender-specific).

Overall survival of first-generation mysids was affected at the 120 ng/L level. After 28 days, survival averaged 75-90% in the controls and ≤60 ng/L groups, and 28% in the 120 ng/L group. The resultant LC_{50} value (with 95% C.I.) was 103.0 (91.3-122.8) ng/L.

Reproductive success (the mean number of young/female/reproductive day) was 1.35, 1.20, 1.28, 1.08, 1.17, 0.98, and 0.0 in the negative control, solvent control, 7.4, 14, 26, 60, and 120 ng/L treatment groups, respectively. The reproductive data were not homogeneous, and no significant differences were observed using non-parametric statistical analyses; however, decreases in reproductive success were observed with increasing chemical concentration, and these differences are probably biologically significant.

The most sensitive endpoint was mean terminal male length, with statistical reductions observed at the 60 and 120 ng/L levels compared to pooled controls. At 28 days, the mean length of surviving first-generation male mysids was 7.6, 7.8, 7.8, 7.4, 7.6, 7.3, and 6.8 mm in the negative control, solvent control, 7.4, 14, 26, 60, and 120 ng/L treatment levels,

respectively. No statistically-significant differences of female length, or male or female dry body weights were observed. However, female length data were not homogeneous, and although no significant differences were observed using non-parametric statistical analyses, slight decreases in female length were observed with increasing chemical concentration, and these differences may be biologically significant. At 28 days, the mean length of surviving first-generation female mysids was 7.7, 7.8, 7.6, 7.4, 7.6, 7.3, and 7.3 mm in the negative control, solvent control, 7.4, 14, 26, 60, and 120 ng/L treatment levels, respectively. The mean dry weight of the surviving first-generation male mysids was 0.83, 0.88, 0.87, 0.86, 0.86, 0.84, and 0.74 mg in the negative control, solvent control, 7.4, 14, 26, 60, and 120 ng/L treatment groups, respectively. The mean dry weight of the surviving first-generation female mysids was 1.1, 1.1, 1.1, 0.96, 1.1, 1.0, and 0.89 mg in the negative control, solvent control, 0.10, 7.4, 14, 26, 60, and 120 ng/L treatment groups, respectively

Based on significant reductions on terminal body length measurements of first-generation male mysids, the NOEC is 26 ng/L, the LOEC is 60 ng/L, and the MATC is 39 ng/L.

Survival data should have been reported daily as well as in terms of each gender, when possible. In addition, the day of first brood release should have been assessed. Because this information was not reported, it was not clear if the test was conducted for the minimum duration specified. Finally, the second-generation mysids should have been observed daily for at least 4 days for survival, development, and behavior. Because these data were not provided, this study, although scientifically valid, does not fulfill the guideline requirements for an aquatic invertebrate life-cycle toxicity test using *Americamysis bahia* [§72-4(c)], and is classified as **Supplemental**.

Results Synopsis:

Survival (Day 28)

LC_{50}: 103.0 ng/L 95% C.I.: 91.3-122.8 ng/L
NOEC: 60 ng/L
LOEC: 120 ng/L
MATC: Not determined

Reproduction (no. young/female/reproductive day)

NOEC: 120 ng/L
LOEC: >120 ng/L
MATC: Not determined

Male length
NOEC: 26 ng/L
LOEC: 60 ng/L
MATC: 39 ng/L

Female length
NOEC: 120 ng/L
LOEC: >120 ng/L
MATC: Not determined

Male dry weight
NOEC: 120 ng/L
LOEC: >120 ng/L
MATC: Not determined

Female dry weight
NOEC: 120 ng/L
LOEC: >120 ng/L
MATC: Not determined

Most sensitive endpoint: Terminal length of males.

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: Daily survival data were not provided, nor were the summarized data following pairing (Day 28) gender-specific; the first day of brood release was not reported so it was not clear if the test was conducted for the minimum duration specified; and second-generation mysids were not observed daily for at least 4 days for survival, development, and behavior.

C. Repairability: Since the second-generation mysids were counted and then discarded, this study is not repairable.

9. GUIDELINE DEVIATIONS:

1. Pretest health and/or mortality of the adult culture was not reported.
2. It was not specified if the flow splitting accuracy within 10%.
3. The amount of solvent used (2.0 mL/L) was much greater than recommended (0.1 mL/L).
4. The time of first brood release was not specified.
5. The material, size, and fill volume of the exposure aquaria were not reported, and it was not reported if the exposure aquaria were identical or were covered during testing.

6. The quantity of live brine shrimp fed to the mysids was not specified.

7. Daily survival and mortality data were not provided for the parental generation.

8. Live offspring were counted and discarded. Survival, development, and behavior of second-generation mysids were not monitored for at least 4 days.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the chronic toxicity of Novaluron to an estuarine/marine shrimp for the purpose of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
Species An estuarine shrimp species, preferably *Americamysis bahia*	*Americamysis bahia*
Source/Supplier	In-house cultures maintained by Springborn.
Age at Beginning of Test <24 hours old	≤24 hours old
Parental Acclimation Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. Mysids should be in good health.	Adult mysids were held in water from the same source as used during the test. Health of the mysid population was not reported.
Parental Acclimation Period At least 14 days	Continuous

Guideline Criteria	Reported Information
Brood Stock Test started with mysids from: - one brood stock, or - brood stock which has not obtained sexual maturity or had been maintained for >14 days in a laboratory with same food, water, temperature, and salinity used in the test.	At test initiation, juvenile mysids were collected from a culture stock that was maintained in the laboratory under the same conditions used in the definitive test.

B. Test System

Guideline Criteria	Reported Information
Source of Dilution Water May be natural (sterilized and filtered) or a commercial mixture; water must be free of pollutants.	Water used for culturing and testing was artificial seawater prepared using freshwater (soft) with a commercially prepared salt formula (hw-MARINEMIX®). Periodic analyses of pesticides, PCB's, and toxic metals in the dilution water indicated the none of these compounds were detected at concentrations that are considered toxic (p. 13).
Does water support test animals without observable signs of stress?	Yes
Water Temperature 27°C for mysids - At test termination, mean-measured temperature for each chamber should be within 1°C of selected test temperature. - Must be within 3°C of the mean of the time-weighted averages. - Must not differ by >2°C between chambers during the same interval.	Target: 26 ± 2°C Actual range: 24.0-27.0°C. - All criteria were met.

Guideline Criteria	Reported Information
Salinity 15-30 ‰ - The difference between highest and lowest measured salinities should be less than 5‰.	23-26‰ Criteria met.
pH 7.6-8.2	7.8-8.2
Dissolved Oxygen 60-100% saturation	5.8-7.6 mg/L (≥78% of saturation).
Photoperiod 16-hr light/8-hr dark (14-hr light/10-hr dark also acceptable)	16 hours light, 8 hours dark.
Test Chambers 1. Material: All glass, No. 316 stainless steel, or perflorocarbon plastic 2. Size: Typically 30 x 45 x 15 cm (20.25 L) 3. Fill depth: 10 cm 4. Were chambers identical and covered during the test?	1. Not reported 2. Not reported 3. Not reported 4. Not reported
Test Compartments (within chambers) - 250-mL glass beakers with side cutouts covered with nylon mesh or stainless steel screen, or - 90- or 140-mm id glass Petri dish bottoms with collars made of 200-250 μm mesh screen	Test compartments were glass petri dishes (10-cm diameter, 2-cm depth) with 15-cm high collars of nylon mesh (350 μm). The solution volume fluctuated from 390 to 710 mL (due to siphon drains). Reproductive compartments (beginning on Day 14) were cylindrical glass jars (5.1-cm diameter, 10-cm height) with two holes of nylon mesh (350 μm) screen. The solution volume fluctuated from 100 to 180 mL.

Guideline Criteria	Reported Information
Type of Dilution System Intermittent flow proportional diluters or continuous flow serial diluters should be used.	A continuous-flow proportional diluter.
Toxicant Mixing 1. Mixing chamber is recommended but not required; aeration should not be used for mixing. 2. If a mixing chamber was not employed, was it demonstrated that the test solution was completely mixed before introduction into the test system? 3. Was flow splitting accuracy within 10%?	1. A continuous mixing chamber was used for the highest test level. 2. N/A 3. Not specified
Flow Rate 1. 5-10 volume additions per 24 hours. 2. Did the flow rate maintain the toxicant level and the DO at ≥60% of saturation? 3. Were the meter systems calibrated before study and checked twice daily during test period?	1. 7.5 volume additions/24 hours 2. Yes 3. Yes
Solvents - Acceptable solvents include triethylene glycol, methanol, acetone, and methanol. - Solvent should not exceed 0.1 mL/L in a flow-through system.	Acetone, 2.0 mL/L
Aeration Dilution water should be vigorously aerated, but the test tanks should not be aerated.	The dilution water was aerated prior to use. The test chambers were not aerated.

C. Test Design

Guideline Criteria	Reported Information
Duration of the Test Approximately 28 days. Was the test terminated within 7 days of the median time of first brood release in the controls?	28 days Unknown. Time of first brood release was not specified.
Nominal Concentrations Negative control, a solvent control (when applicable), and at least five treatment levels, one of which must adversely affect a life stage and one must not affect any life stage. The dilution factor should not be >50%.	0 (negative and solvent controls), 8.2, 16, 33, 65, and 130 ng/L
Distribution Number of mysids before pairing: Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/treatment level. Number of mysids after pairing: ≥20 randomly selected pairs/treatment (excess males should be held in separate compartment in same treatment to replace paired males).	60 mysids/level: 15 mysids/compartment, 2 compartments/aquarium, and 2 replicate test aquaria/level. 20 pair/level: 1 pair/compartment, 10 compartments/aquarium, and 2 replicate test aquaria/level. Extra mysids were pooled and placed in one of the initial retention compartments within each aquarium; male mysids from this pool were used to replace dead males from the paired groups.
Pairing Should be conducted when most of the mysids are sexually mature, usually 10-14 days after test initiation. All pairing should occur on the same day.	Female and male adults were paired on Day 14 and reproduction was monitored through Day 28.
Test organisms randomly or impartially assigned to test vessels?	Yes

Guideline Criteria	Reported Information
Were treatments randomly assigned to individual test chamber locations?	Yes
Feeding Mysids should be fed live brine shrimp nauplii at least once daily. 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.	Mysids were fed live brine shrimp (*Artemia salina*) nauplii 2 times/day (amount not reported). The brine shrimp nauplii were enriched with Selco® (substance high in fatty acids) once per week prior to pairing and every-other-day after pairing.
Counts Live adult mysids should be counted at initiation, at pairing, and daily after pairing. Live young must be counted and removed daily. Missing or impinged animals should be recorded.	Yes Yes Yes
Controls Negative control and carrier control (when applicable) are required.	A negative (saltwater) control and solvent (acetone) control was used.
Water Parameter Measurements 1. Temperature should be monitored daily in one chamber and at least three times in all chambers. 2. Salinity should be measured daily in at least one test vessel. 3. pH should be measured at the beginning, the end, and at least weekly during the test in the control vessels and highest test level. 4. Dissolved oxygen must be measured at each concentration at least once a week.	1. Temperature was measured in each replicate chamber daily, and continuously in one solvent control chamber. 2. Salinity was measured in each replicate chamber daily. 3. The pH was measured in each replicate chamber daily. 4. DO was measured in each replicate chamber daily.

Guideline Criteria	Reported Information
Chemical Analysis Toxicant concentration must be measured in one chamber at each toxicant level every week.	Water samples were collected from each test chamber on Days 0, 7, 14, 22, and 28. Samples were analyzed for total radioactivity using LSC. In addition, Rimon (Novaluron) concentrations were confirmed in samples from the highest treatment level (130 ng/L) using HPLC/RAM analysis.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Chemical Analysis For all test groups, a) the measured concentration of the test material should not be <50% of the time-weighted average measured concentration for >10% of the duration of the test, and b) the measured concentration should not be >30% of the time-weighted average measured concentration for >5% of the duration of the test.	All criteria met. Based on LSC analysis, mean-measured concentrations were <0.39 (LOQ, controls), 7.4, 14, 26, 60, and 120 ng/L, and are 80 to 92% of the nominal concentrations (Table 2, p. 30). HPLC results supported LSC results, confirming the stability of Rimon under test conditions. Based on HPLC analysis, [^{14}C]Rimon accounted for 94.8 to 100% of the total radioactivity recovered in the high-dose solutions (Table 3, p. 31).

Guideline Criteria	Reported Information
Controls - Survival of the paired first-generation controls must be ≥70%. - ≥75% of the paired first-generation female controls produced young, or - The average number of young produced by the first-generation female controls was ≥3.	All criteria met. Survival of paired first-generation negative and solvent controls were both 77%. The average number of young produced by the first-generation female controls was ≥3.
Data Endpoints Must Include 1. Survival of first-generation mysids, gender specified 2. Number of live young produced per female 3. Dry weight and length of each first generation mysid alive at the end of the test, gender specified **Data Endpoints Should Also Include** 4. Incidence of morphological findings. 5. Survival, development, and behavior of second-generation mysids for at least 4 days.	1. Survival of first-generation mysids at study termination (Day 28; combined sexes) 2. Number of live young produced per female per reproductive day (Day 28). 3. Dry weight and length of each first generation living at end of test; data were gender-specific. 4. Abnormal appearance or behavior of first-generation mysids. 5. Criteria NOT met. Except for survival, toxic effects of second-generation mysids were not addressed in the study.
Raw data must include 1. Survival of first-generation mysids, gender specified 2. Number of live young produced per female 3. Terminal weight and length measurements, individual and gender specified	1. Criteria NOT met. Summarized data were provided for terminal (Day 28) survival; data were not gender-specific. 2. Criteria met. 3. Criteria met.

Effects Data

Concentration (ng/L)		Survival Day 28[1]			Reproduction, Days 14-28		Growth, Day 28			
Nominal	Mean Measured (% nominal)	No. ♂	No. ♀	Percent (ratio) ♂ and ♀	Total No. of Young	Mean No. Young/Female/ Day	Mean Length, mm		Mean Dry Weight, mg	
							♂	♀	♂	♀
Control	<LOQ	15	31	77 (46/60)	345	1.35	7.6±0.41	7.7±0.41	0.83±0.12	1.1±0.19
Solvent Control	<LOQ	26	20	77 (46/60)	331	1.20	7.8±0.34	7.8±0.33	0.88±0.09	1.1±0.22
8.2	7.4 (90)	23	24	78 (47/60)	356	1.28	7.8±0.47	7.6±0.47	0.87±0.14	1.1±0.20
16	14 (85)	24	27	85 (51/60)	301	1.08	7.4±0.54	7.4±0.29*	0.86±0.11	0.96±0.16
33	26 (80)	19	26	75 (45/60)	321	1.17	7.6±0.33	7.6±0.32	0.86±0.11	1.1±0.17
65	60 (92)	22	33	90 (55/60)	261	0.98*	7.3±0.28*	7.3±0.34*	0.84±0.12	1.0±0.20
130	120 (89)	4	13	28* (17/60)	0	0.00[2]	6.8±0.41[2]	7.3±0.48[2]	0.74±0.06[2]	0.89±0.15[2]

NR=Not reported

[1] The number of surviving organisms/sex was reviewer-derived from raw terminal growth data tables (pp. 77-80). Only combined-sex data were evaluated statistically.

[2] Data in the 120 ng/L treatment group were not statistically analyzed at these endpoints due to significant effects on survival.

*Determined to be significantly different from the pooled controls ($p \leq 0.05$) by the study authors using Williams' or Bonferonni's Test.

Toxicity Observations: First-generation mysids were reportedly observed daily for abnormal appearance and behavior. No discussion of findings (if any) was provided.

Survival of first-generation mysids was statistically-reduced at the 120 ng/L level compared to pooled controls, and the terminal length of the first-generation mysids (both sexes) was statistically-reduced at the 60 ng/L level. Although a statistically-significant reduction in terminal length was observed in females at the 14 ng/L level, since a similar effect was not observed at the next higher level, this effect was not considered to be treatment related (p. 25). Due to the significant effect on survival, terminal growth measurements were not statistically analyzed at the 120 ng/L level.

The number of offspring/female/reproductive day ("reproductive success") was statistically-reduced at the 60 ng/L level. Due to the significant effect on survival, reproductive success was not statistically analyzed at the 120 ng/L level.

B. Statistical Results:

Statistical analyses were performed on terminal (Day 28) survival of the first-generation mysids, the number of young released per female per reproductive day, and the terminal length and dry weight of each surviving first-generation mysid. Data were analyzed by standard statistical techniques using a computer program (West, Inc., and Gulley, 1994). A T-test determined there were no differences between the negative and solvent control data for each endpoint, so the controls were pooled for analysis. The Shapiro-Wilk's Test was used to determine that data were normally distributed, and Bartlett's Test was used to determine that variances were homogeneous. For each endpoint, the performance of organisms exposed to each treatment level of the test substance was compared with the performance of the pooled control using the Williams' Test or Bonferroni's Test. Analyses were conducted at the 95% level of certainty, except for the Bartlett's Test and Shapiro-Wilk's Test, in which the 99% level of certainty was applied. Mean measured values were used in all estimations.

Most sensitive endpoint: Reproductive success and terminal length (both sexes)

Results Synopsis

Endpoint	Method	NOEC	LOEC	MATC
Adult Survival (Day 28)	Williams' Test	60 ng/L	120 ng/L	N/D
Reproduction (mean no. young/female/reproductive day)	Williams' Test	26 ng/L	60 ng/L	39 ng/L
Female Length (mm)	Bonferroni's Test	26 ng/L	60 ng/L	39 ng/L
Male Length (mm)	Williams' Test	26 ng/L	60 ng/L	39 ng/L
Female Dry Weight (mg)	Bonferroni or Williams' Tests	60 ng/L	>60 ng/L	N/D
Male Dry Weight (mg)	Bonferroni or Williams' Tests	60 ng/L	>60 ng/L	N/D

N/D - Not determined

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical analyses were verified for percent survival of the first-generation mysids (Day 28), the number of young released per female per reproductive day, and the terminal length and dry weight of each surviving first-generation mysid (gender specific). The NOEC and LOEC for percent survival were visually determined. The LC_{50} (with 95% confidence intervals) was determined using the moving average angle method via TOXANAL statistical software. Data for reproductive success, female length, and female dry weight were normally distributed, but the variances were heterogeneous. As a result, the NOEC and LOEC for these endpoints were determined using the non-parametric Kruskal Wallis test followed by Dunn's multiple comparison test. Data for male length and male dry weight were normally distributed and the variances were homogeneous. The NOEC and LOEC for these endpoints were determined using ANOVA followed by William's test via TOXSTAT statistical software. The reviewer based all estimates on the mean-measured concentrations. The Student's t-test determined there was no difference in the negative and solvent controls, so the controls were pooled for all comparisons.

Most sensitive endpoint: Terminal length of males.

Results Synopsis

Endpoint	Method	NOEC	LOEC
Adult Survival (Day 28)	Visually determined	60 ng/L	120 ng/L
Reproduction (mean no. young/female/reproductive day)	Kruskal Wallis/Dunn's	120 ng/L	>120 ng/L
Female Length (mm)	Kruskal Wallis/Dunn's	120 ng/L	>120 ng/L
Male Length (mm)	Williams' Test	26 ng/L	60 ng/L
Female Dry Weight (mg)	Kruskal Wallis/Dunn's	120 ng/L	>120 ng/L
Male Dry Weight (mg)	Williams' Test	120 ng/L	>120 ng/L

14. REVIEWER'S COMMENTS:

The reviewer's estimates for reproductive success and female length differed from the study author's because the study author inappropriately determined these values using the parametric William's and Bonferroni's tests. Based on the reviewer's statistical analysis (which are less sensitive), no differences between the control and test groups were observed for these endpoints; however, decreases in reproductive success and female length were observed with increasing chemical concentration, and these differences are probably biologically significant. The reviewer's conclusions agreed with the study author's with respect to survival, male length, and male and female dry weights. Unlike the study author, however, the reviewer included data from the 120 ng/L treatment level in all statistical comparisons. The reviewer's findings are reported in the Conclusions sections.

Several significant deviations from §72-4c guidance were observed: daily survival and mortality data of the first-generation mysids were not provided (and reported in terms of each gender when possible); the first day of brood release was not reported; and second-generation mysids were not observed daily for at least 4 days for survival, development, and behavior. As a result, this study does not fulfill guideline requirements for an aquatic invertebrate life-cycle toxicity test using *Americamysis bahia* [§72-4(c)]. This study is therefore classified SUPPLEMENTAL.

HPLC/RAM characterization was only performed from samples collected at the highest test level of 120 ng/L, and demonstrated that [^{14}C]Rimon was stable under test conditions (HPLC recoveries correlated with LSC recoveries). Based on the HPLC analyses provided, Rimon accounted for 94.8-100% of the radioactive distribution (Table 3, p. 31).

Although the solvent (acetone) concentration (2.0 mL/L) greatly exceeded the limit of 0.1 mL/L, this did not appear to have an adverse affect on the mysid population, and this deviation from guidelines is considered minor. However, no explanation was provided by the study author as to why such a high level of solvent was used.

Similarly, although the feeding rate for the mysids was not specified, the amount offered appeared to be sufficient and did not appear to have an adverse effect on any biological response (i.e., survival, growth, reproduction, lack of cannibalistic behavior).

Throughout the exposure period, no visible sign of undissolved test substance was observed in the mixing chamber, the chemical cells of the diluter system, or in any of the exposure solutions (pp. 22-23).

The TOC concentrations of the dilution water were 0.36 and 0.63 mg/L for November and December 2001, respectively.

This study conformed with Good Laboratory Practice Standards as published by the U.S. EPA GLP Regulations (40 CFR, Part 160) with the following exception: routine food and water contaminant screening analyses for pesticides, PCBs, and toxic metals were not collected in accordance with GLP procedures (p. 3). A Quality Assurance Statement was provided.

15. REFERENCES:

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16. RESULTS OF STATISTICAL VERIFICATION:

LC50
Moving average angle method

		LC50	95% CI	
1	.1077595	**102.9013**	**91.32477**	**122.8119**

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	11.03326	13.62183	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = .7238091
95 PERCENT CONFIDENCE LIMITS =-1.680421 AND 3.128039

LC50 = 375.4478
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 6.606076
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

reproductive success
File: 8212r Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	1.275	1.275	43.500
2	7.4	1.300	1.300	23.000
3	14	1.050	1.050	10.500
4	26	1.150	1.150	15.500
5	60	0.970	0.970	9.500
6	120	0.000	0.000	3.000

Calculated H Value = 10.480 Critical H Value Table = 11.070
Since Calc H < Crit H **FAIL TO REJECT Ho:All groups are equal.**

reproductive success
File: 8212r Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 5 3 4 1 2
6	120	0.000	0.000	\
5	60	0.970	0.970	. \
3	14	1.050	1.050	. . \
4	26	1.150	1.150	. . . \
1	GRPS 1&2 POOLED	1.275	1.275	. . . . \
2	7.4	1.300	1.300	. . . . . \

```
* = significant difference (p=0.05)              . = no significant difference
Table q value (0.05,6) =   2.936          Unequal reps - multiple SE values
```

male body length
File: 8212ml Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.469	0.294	10.889
Within (Error)	8	0.220	0.027	
Total	13	1.689		

Critical F value = 3.69 (0.05,5,8)
Since F > Critical F REJECT Ho:All groups equal

male body length
File: 8212ml Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	7.700	7.700		
2	7.4	7.750	7.750	-0.351	
3	14	7.450	7.450	1.757	
4	26	7.600	7.600	0.703	
5	60	7.350	7.350	2.460	
6	120	6.750	6.750	6.676	*

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

male body length
File: 8212ml Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	7.4	2	0.412	5.4	-0.050
3	14	2	0.412	5.4	0.250
4	26	2	0.412	5.4	0.100
5	60	2	0.412	5.4	0.350
6	120	2	0.412	5.4	0.950

male body length
File: 8212ml Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	7.700	7.700	7.717
2	7.4	2	7.750	7.750	7.717
3	14	2	7.450	7.450	7.525
4	26	2	7.600	7.600	7.525
5	60	2	7.350	7.350	7.350
6	120	2	6.750	6.750	6.750

male body length
File: 8212ml Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	7.717				
7.4	7.717	0.116		1.86	k= 1, v= 8
14	7.525	1.219		1.96	k= 2, v= 8
26	**7.525**	**1.219**		**2.00**	**k= 3, v= 8**
60	7.350	2.437	*	2.01	k= 4, v= 8
120	6.750	6.615	*	2.02	k= 5, v= 8

s = 0.166
Note: df used for table values are approximate when v > 20.

female length
File: 8212fl Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	7.775	7.775	49.000
2	7.4	7.600	7.600	17.500
3	14	7.450	7.450	11.000
4	26	7.600	7.600	17.000
5	60	7.350	7.350	6.500
6	120	7.300	7.300	4.000

Calculated H Value = 11.712 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

female length
File: 8212fl Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 5 3 2 4 1
6	120	7.300	7.300	\
5	60	7.350	7.350	. \
3	14	7.450	7.450	. . \

2	7.4	7.600	7.600	. . . \
4	26	7.600	7.600	. . . . \
1	GRPS 1&2 POOLED	7.775	7.775	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

male body weight
File: 8212mw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.015	0.003	1.000
Within (Error)	8	0.021	0.003	
Total	13	0.036		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

male body weight
File: 8212mw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	0.852	0.852		
2	7.4	0.865	0.865	-0.264	
3	14	0.855	0.855	-0.053	
4	26	0.865	0.865	-0.264	
5	60	0.835	0.835	0.369	
6	120	0.765	0.765	1.845	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

male body weight
File: 8212mw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	7.4	2	0.137	16.1	-0.013
3	14	2	0.137	16.1	-0.003
4	26	2	0.137	16.1	-0.012
5	60	2	0.137	16.1	0.017
6	120	2	0.137	16.1	0.087

male body weight
File: 8212mw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	0.852	0.852	0.858
2	7.4	2	0.865	0.865	0.858
3	14	2	0.855	0.855	0.858
4	26	2	0.865	0.865	0.858
5	60	2	0.835	0.835	0.835
6	120	2	0.765	0.765	0.765

male body weight
File: 8212mw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	0.858				
7.4	0.858	0.124		1.86	k= 1, v= 8
14	0.858	0.124		1.96	k= 2, v= 8
26	0.858	0.124		2.00	k= 3, v= 8
60	0.835	0.393		2.01	k= 4, v= 8
120	0.765	1.966		2.02	k= 5, v= 8

s = 0.051
Note: df used for table values are approximate when v > 20.

female body weight
File: 8212fw Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	1.075	1.075	39.000
2	7.4	1.050	1.050	17.000
3	14	1.005	1.005	14.000
4	26	1.050	1.050	17.000
5	60	1.025	1.025	15.000
6	120	0.890	0.890	3.000

Calculated H Value = 6.385 Critical H Value Table = 11.070
Since Calc H < Crit H **FAIL TO REJECT Ho:All groups are equal.**

female body weight
File: 8212fw Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 3 5 2 4 1
				- - - - - -

6	120	0.890	0.890	\
3	14	1.005	1.005	. \
5	60	1.025	1.025	. . \
2	7.4	1.050	1.050	. . . \
4	26	1.050	1.050	. . . . \
1	GRPS 1&2 POOLED	1.075	1.075	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

evans novaluron 28 day marine invertebrate lc50

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
120	46	30	65.2174	0
60	60	6	10	0
26	46	1	2.1739	0
14	60	9	15	0
4.4	60	13	21.66667	0

THE BINOMIAL TEST SHOWS THAT 60 AND 120 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 100.8869

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.1000057	100.8869	90.05301	118.6461

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	15.56625	15.68014	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = .573484
95 PERCENT CONFIDENCE LIMITS =-1.689145 AND 2.836113

LC50 = 621.5929
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 3.792903
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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